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NURSERY CONIFER DISEASES

evaluation of Benomyl to control root disease of Engelmann spruce seedlings

FOREST ENVIRONMENTAL PROTECTION
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Cover photo: Application of benomyl to nursery beds.

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NURSERY CONIFER DISEASES - EVALUATION OF BENOMYL TO CONTROL ROOT DISEASE OF ENGELMANN SPRUCE SEEDLINGS 1/

by

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ABSTRACT

The benomyl fungicide, Tersan, applied at 7 and 14 pounds active ingredient per acre, on April 26 and July 25, 1973, was ineffective in reducing mortality in 2-0 Engelmann spruce seedlings derived from two different seed sources growing in three different nursery sections at the USFS Coeur d'Alene Nursery.

Percent mortality in plots monitored over the entire summer was 8.6, 3.6 and 8.0 in sections 31, 36, and 37 respectively, and ranged from 0 to as high as 21.9 in individual mortality plots. Percent mortality in sections 31 and 37 was similar and significantly greater than in section 36, indicating variation in conditions associated with individual sections. Percent mortality combined for sections 36 and 37 (seed source 72-180) was significantly less than that for section 31 (seed source 72-144) indicating variation in host or seed lot susceptibility or tolerance.

Higher rates of application, 28 and 42 pounds active per acre, applied during a second treatment period were ineffective. Because of experimental design inadequacies, however, the results are inconclusive.

Bioassay studies to determine chemical distribution in soil and within seedlings were generally negative. Reasons for lack of control and negative assays are discussed.

Isolation studies did reveal a high frequency of occurrence of *Fusarium* spp. in dead seedlings, and disease expression increased with increasing summer temperatures, implicating fusarial pathogens in the causal complex.

INTRODUCTION

During the fall of 1972, Steve McDonald, nurseryman at the USDA Forest Service tree nursery in Coeur d'Alene, Idaho, reported a serious problem on 5 acres of 1-0 Engelmann spruce. Nearly 80% of the seedlings exhibited

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1/ This paper reports work involving chemical fungicides. It does not include recommendations for their operational use nor does it imply that uses discussed here have been registered. All uses of pesticides must be registered by appropriate State or Federal agencies or both before they can be recommended.

needle necrosis, dead tops, and/or general chlorosis. The unhealthy appearance was diagnosed by Clinton Carlson, Region 1 Pathologist, as being caused by a combination of factors including winter damage, frost damage, root rot, and soil characteristics which may or may not have been interrelated.

These seedlings were urgently needed for reforestation of cutover areas in both Regions 1 and 4. The objectives of this study were to evaluate the effectiveness of the benomyl fungicide Tersan 2/ in saving root-diseased seedlings, and preventing infection of stressed seedlings.

Tersan 2/, a benomyl fungicide hereafter referred to as benomyl (methyl 1-(butyl carbamyl)-2-benzimidazole carbamate), was the material selected for the following reasons:

1. Benomyl is a systemically active material and has been shown to provide both curative and preventative control of field drop diseases caused by several formae specialis of *Fusarium oxysporum* Schlect. emend. Snyd. and Hans. (Biehn and Dimond, 1971; Erwin, 1973).

2. Benomyl has the widest spectrum of fungitoxic activity of all the newer systemics (Erwin, 1973).

3. Use of benomyl to control root disease in western white pine and in Douglas-fir has apparently been successful. 3/

4. Phytotoxocity to benomyl is low (Anonymous, 1969).

Rates used were based mainly on recommendations of D. W. Finnerty, field development biologist for E. I. duPont de Nemours and Company.

MATERIALS AND METHODS

Experimental Design

Twenty-six spray plots were located within three nursery sections occupied by two different seed sources.

Sections are laid out with the long axis running east to west, and are composed of nine beds, each including seven drill rows. Sections and beds are numbered systematically from north to south.

Two plots were randomly located in each of five seedbeds in section 31, (beds 5 through 9) containing seedlings from Region 1 seed source designated 72-144 and two in each of eight beds in section 36 (beds 5 through 9); and

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2/ Registered trade name of E. I. duPont deNemours and Company, Inc. Mention of commercial products is for convenience only and does not imply endorsement by the U.S. Department of Agriculture.

3/ Personal communication with Steve McDonald, Western Nursery and Greenhouse Specialist, USDA Forest Service, Lakewood, Colorado.

section 37 (beds 1 through 3) containing seedlings from a Payette National Forest (Region 4) seed source designated 72-180.

Initially each spray plot consisted of three adjacent subplots defined as a 10-foot linear section of the seedbed, encompassing all seven drill rows. Two additional subplots were added to some plots for a second spray application. A 2-foot buffer zone was added to each end of each subplot.

A 1-foot-square mortality plot referenced by wooden dowels, was randomly established within each 10-foot spray plot, excluding the outer drill row on each side. Before treatment, all dead seedlings were removed from each mortality plot and live seedlings counted.

Chemical Application

The first chemical application was made April 26, 1973. Temperatures were mild and wind velocity was 5 to 7 miles per hour across seedbeds. The evening prior to spraying, beds were thoroughly sprinkled, to provide a solvent front for chemical movement.

Of the three subplots within each plot, one received benomyl at the rate of 14 pounds active material per acre, one at the rate of 7 pounds active material per acre, and one with none. In all cases, the sequence of subplots beginning from the west end was 14 pounds, 0 pounds, and 7 pounds.

On July 25, benomyl was again applied at the same rates to the same subplots except for one plot in each bed in section 31. Plots were left unsprayed to provide a second check as to chemical effectiveness. Additionally two subplots, one receiving chemical at 28 pounds and one at 42 pounds active material per acre were established where space permitted at the east end of each plot. This amounted to 19 new subplots treated at each rate.

An aqueous solution/suspension of benomyl was mixed immediately prior to spray time, and applied at indicated dosages with a tractor-mounted spray system. Spray booms were maintained at 12 to 18 inches above ground at all times to insure complete coverage and to reduce drift. An electric agitator was used inside the spray tank to keep the highly insoluble chemical material suspended.

Postspray Sampling

Following the first application, on the fourth and twentieth days after chemical application and at approximately 3½-week intervals thereafter until July 25, and on the fifth, twentieth and fiftieth days following the second application, living and dead seedlings in all mortality plots were counted. On each date all dead seedlings from inside each mortality plot were removed keeping as much of the root system intact as possible. Except for the three dates following the second application,

five live seedlings were obtained from outside each mortality plot. Dead and live trees from each mortality plot were maintained separately in plastic bags. Samples were placed in a portable cooler in the field, then packaged and sent to Missoula for laboratory evaluation. Since little mortality was evident at the first two sampling dates, several top-killed and several dead seedlings adjacent to mortality plots were removed from each of the two seedlots, and processed as above.

Soil samples, one from each treatment or check in each seedlot were obtained with a 2.5 cm diameter soil sampler from the central portion of respective plots at each of the above dates. Each sample was carefully placed into a separate plastic bag, wrapped so as to maintain integrity of the core, and sent to Missoula for laboratory studies.

Laboratory Evaluations

Fungal isolations were made from live and dead seedlings as follows: Seedlings were thoroughly washed in tap water to remove dirt and foreign matter, dipped in alcohol and flamed for 1 to 2 seconds. Segments approximately 2 cm long were excised from: (1) just above root collar; (2) just below root collar; (3) half way down root system; and (4) root tip, and placed into petri plates containing 25 milliliters (ml) standard potato dextrose agar (PDA) to which streptomycin sulfate had been added at the rate of 0.01 gram (gm)/liter (1). Plates were incubated in the dark at 25° C for 1 to 2 weeks and then evaluated for fungal growth.

Preliminary evaluations of bioassay methods indicated that *Fusarium oxysporum* could be used as a bioassay organism to detect benomyl at rates equivalent to 7 lbs active/acre on circular paper assay discs. Thus, *F. oxysporum* was used in the bioassay system to determine whether quantities of chemical sufficient to affect the suspected pathogen were absorbed by the seedlings.

Healthy seedlings from treated and untreated plots were washed for 10 to 15 minutes in running tap water, dipped in alcohol and flamed for 1 to 2 seconds. One cm long assay chips were aseptically cut from the upper root and lower stem and placed on assay petri plates containing 10 ml of standard PDA, on which sterile 1-cm diameter assay discs, one saturated with sterile distilled water (control) and one containing a quantity of an aqueous solution/suspension of benomyl equivalent to the 7 lbs per acre treatment. The surface of the agar including assay chips and discs was then covered with 10 ml of molten seed agar comprised of standard PDA containing approximately 500,000 *Fusarium oxysporum* 4/ propagules per ml After cooling, plates were placed upside down in a refrigerator (2-5° C) for 24 hours, to allow for chemical diffusion into the seed agar. Following the diffusion period, plates were incubated at 25° C until spore germination occurred and then examined for inhibition zones.

4/ *Fusarium oxysporum* had been isolated from dead Douglas-fir seedlings previously untreated with benomyl from the Coeur d'Alene Nursery.

A 1-cm section of soil was taken from the top and from 3 and 6 cm down, on each soil sample. Each portion was maintained separately, thoroughly mixed, and allowed to air-dry overnight. From each of these portions, 0.5 gm of soil was placed into a depression cut in the center of petri plates containing 10 ml of the *Fusarium* seed agar. To each sample 0.05 ml sterile distilled water was added. Assay discs were placed on the agar surface and plates treated as for the seedling assays.

Data Analyses

Percent mortality rather than actual mortality and the 95% level of significance were used in all analyses.

Initially, individual mortality plot data was analyzed separately for each spray date. Because chemical effects in terms of mortality reduction would not have been obvious, data from the first sampling date following the second spray application was grouped with data from the first spray date.

Several different statistical tests were used to evaluate mortality included for the first spray date. Differences in mortality between overall treatment rates and between individual mortality plots were subjected to variance analysis. Paired or unpaired t-tests were employed to determine whether significant differences in percent mortality existed between sections, between seed sources, or between individual treatments within a section or between two sections.

Unpaired t-tests were used to test data included for the second spray date.

Percent sampling error, i.e.:

$$\frac{\text{standard error of the mean}}{\text{estimated mean}} \times t \text{ value at selected level of confidence} \times 100,$$

(Wear et al., 1966) was calculated at the 95% level of confidence and was used to indicate reliability of mortality plot information for both spray dates.

Data inspection indicated differences in mortality by date and empirical field examinations indicated higher mortality in beds closest to sprinklers (beds 1 and 9) than in interior beds (2 through 8). To test these differences, mean percent mortality values of each treatment for each sampling date within the two bed groupings for each section were subjected to variance analysis.

Percent isolation frequency of probable pathogens (*Fusarium* spp. and *Cylindrocarpon* sp.) from healthy as well as dead seedlings was determined for each treatment at each evaluation date. Analysis of variance tests was applied to this data to determine whether percent isolation frequency differences between treated or untreated seedlings, and between healthy or dead seedlings at different sampling dates or from either seedlot were significant.

RESULTS

Field Evaluations

Analysis of data included for the first spray date (Appendix I) indicated no significant difference in percent mortality between treatments or between individual mortality plots (Table 1). Percent mortality for individual mortality plots ranged from 0 percent to 21.9 percent (Appendix I).

Table 1.--Analysis of variance for evaluation of effects of initial benomyl application

Source	Degrees of freedom	Sums of squares	Mean squares	F value
Total	77	1,808.24000	23.48360	
Treatment	2	9.25923	4.62962	0.100721 n.s.
Mortality plots	25	1,149.12000	45.96460	1.000000 n.s.
Residual	50	649.86100	12.99720	

Unpaired t-tests indicated that percent mortality in sections 31 and 37 was not significantly different, but was significantly greater in each than in section 36, being 6.9%, 2.2%, and 6.3% for sections 31, 36, and 37 respectively.

Grouping sections according to seed source, significantly greater percent mortality was found in seed source 72-144; i.e., section 31, than in seed source 72-180; i.e., sections 36 and 37. Treatment differences between sections were nonsignificant indicating the absence of treatment:section interaction.

Percent sampling error was low for section 36 (15-18%), medium (29-45%) for section 31, and high (82-121%) for section 37 (Appendix I).

Unpaired t-tests detected no significant difference in percent mortality between rates, sections, or between plots sprayed or unsprayed the second date. Following the second treatment, percent mortality by section ranged from 1.4 to 1.8% (Appendix I).

Percent sampling error varied considerably for each treatment:section combination, but was generally lower in checks than in plots receiving chemical (Appendix I).

Differences in percent mortality between dates and between sections were significant, while those between bed groupings were nonsignificant (Table

2). A gradual increase in percent mortality for all beds from April 26 through July 30 and gradual decline thereafter was noted with average percent mortality per mortality plot by date being: 4/30, 0; 5/16, 0.27; 6/6, 1.22; 6/29, 1.0; 7/30, 2.41; 8/14, 0.95; 9/13, 0.76.

First order interactions were nonsignificant.

Table 2.--Analysis of variance for evaluating percent mortality, differences between bed grouping (1 and 9, and 2 through 8), dates, sections, and treatments

Source	Degrees of freedom	Sums of squares	Mean squares	F value
Total	107	232.09000	2.16906	
Sections	2	19.79570	9.89787	5.92106*
Dates	5	46.10160	9.22031	5.51574*
Treatments	2	7.62963E-2	3.81482E-2	2.28208E-2
Bed groupings	1	3.96750	3.96750	2.37342
Residual	97	162.14900	1.67164	

*Significant at 95% level of confidence.

Laboratory Evaluations

Identification of 21 representative *Fusarium* isolates thought to be pathogens involved were confirmed or made by Dr. William Snyder's laboratory at the University of California, Berkeley, California. Three species of *Fusarium* were identified. These were: *Fusarium roseum*, Lk. ex Fr. emend. Snyd. and Hans., *F. oxysporum*, and *F. tricinctum* (Cda.) Sacc.

Fusarium oxysporum and *F. roseum* were frequently isolated, while *F. tricinctum* and a species of *Cylindrocarpon* were infrequently found (Appendix II).

Several unidentified nonsporulating dematiaceous fungi as well as *Alternaria* sp., *Stemphyllium* sp., and *Ulocladium* sp. were commonly isolated, while *Trichoderma* sp., *Penicillium* sp., *Cladosporium* sp., and *Aspergillus* sp. as a group were infrequently isolated (Appendix II).

Data analysis showed no differences in isolation frequency of *Fusarium* spp. and/or *Cylindrocarpon* sp. between treated or untreated healthy seedlings, between healthy or dead seedlings at different sampling dates from either seedlot. Healthy seedlings yielded significantly less of the three fungi than did dead seedlings.

Differences between frequency of isolation of the three fungi from dead seedlings from either seedlot or any date were not significant (Appendix II). Except for occasional slight inhibition zones around soil treated at the 42 pounds/acre rate, soil and seedling bioassays were all negative.

DISCUSSION

Percent seedling mortality was significantly greater in beds sown with R-1 seed source 72-144 (section 31), than in beds sown with R-4 seed source 72-180 (sections 36 and 37). Within seed source 72-180, significantly greater percent mortality was found in section 37 than in section 36.

Variation in percent mortality between individual mortality plots was great and undoubtedly accounted for the lack of significant difference between individual mortality plots.

In all the above cases, genetic factors affecting seedling susceptibility as well as environmental factors affecting one or more of the following (1) pathogen population levels (inoculum density); (2) ability of pathogen to incite disease (capacity); and (3) ability of seedling to contract disease (proneness) are responsible for variation in percent mortality.

In equation form (Baker, 1968) it may be expressed as:

Disease severity = (inoculum density x capacity)
x (proneness x susceptibility).

Unfortunately, none of these factors were defined in these studies and conclusions regarding their interactions as well as interactions with benomyl cannot be made.

Two possible reasons for the apparent ineffectiveness of benomyl are: (1) method of evaluating efficacy was not of sufficient precision to detect effects; or (2) the chemical was truly ineffective. Medium to high percent sampling errors in the first treatment evaluation for each treatment:section combination in sections 31 and 37 (Appendix I) suggest that the large variation in percent mortality between individual mortality plots may have masked any treatment effects. However, low percent sampling errors for combinations in section 36 would indicate that sampling was adequate in that section and that benomyl was truly ineffective.

Future studies should include elimination of one or more sources of variation, and/or greater numbers or different types, sizes, or arrangements of plots should be used.

If, in fact, benomyl was ineffective in these studies, it may have been for one or more of the following reasons:

1. The chemical did not reach the absorbing area in sufficient quantity to be effective against the pathogen(s) or to be detected in the bioassay.

2. The chemical was not actively taken up by roots in sufficient quantities to be effective against the pathogen(s).

3. The chemical was effective for a very short period (a few days) and became ineffective as titer of the material was depleted.

4. Mutants tolerant of the applied levels evolved and multiplied.

Evaluation of various reports in the literature (Bartels-Schooley and MacNeill, 1971; Biehn and Dimond, 1970; Peterson and Edgington, 1970; Hine et al., 1969; Edgington et al., 1971; Pitblado and Edgington, 1972; Leach et al., 1969; Fuchs et al., 1970; and Erwin, 1973) indicate that the first alternative above is the most logical.

As expected for *Fusarium* spp. increased activity comes with warmer weather. Although differences in frequency of isolation of *Fusarium* spp. from seedlings at various sampling dates were not significant, mortality increased significantly from May to late July. This is consistent with studies by Bloomberg (1973).

If mortality is related to *Fusarium* spp., increased mortality may be due to the fact that the fungus is present, but dormant during cooler weather, becoming more virulent as temperatures rise and also that the plant may become more susceptible during warmer weather. As a class, vascular fusaria are favored by relatively high soil temperatures, and in some cases it is possible to grow hosts in cool, infected soil without sufficient infection to produce symptoms (Walker, 1950).

The consistent isolation of *Fusarium* spp. from dead seedlings adds further support to the supposition that *Fusarium* spp. are the primary pathogenic agents involved. Relatively high infection levels found in apparently healthy seedlings are consistent with findings of Bloomberg (1966) and Carlson (1973). However, Bloomberg (1971) indicates that infection does not necessarily lead to mortality. Rather, different strains varying in virulence are present and change within the host brought about by the environment probably stimulates more virulent strains to incite disease.

The possibility of two modes of pathogenesis or parasitism occurring, both contributing to mortality, exists. *Fusarium oxysporum* is a well known vascular pathogen (wilter) causing plugging of xylary elements, imposing a moisture stress on the upper portion of the plant (Walker, 1950). *Fusarium roseum* is often thought of as a rotter, breaking down tissue (Dickson, 1956). Since both organisms were frequently isolated, perhaps *F. oxysporum* is stressing the plant enough to allow *F. roseum* to invade the host, decaying roots, which is so characteristic of dead seedlings found in the Coeur d'Alene Nursery.

CONCLUSIONS

Results of this study indicate that benomyl applied at rates of 7 and 14 lbs. active/acre was ineffective in reducing seedling mortality

apparently caused by *Fusarium* spp. Higher rates, 28 and 42 lbs active/acre were probably not adequately tested since application was made late in the summer, and isolations from healthy seedlings to determine curative effects were not made.

Control of several closely related pathogens has been achieved with benomyl (Erwin, 1973) and the material should not be discarded as totally ineffective. We should recognize its potential and learn from these and other studies how it can be more effectively used. For example:

1. Benomyl has been shown to move much further in soil if certain adjuvants are added to the chemical mixture (Pitblado and Edgington, 1972);

2. Benomyl has a somewhat selective spectrum and is effective against fusaria (Deuteromycetes) but often not against Phycomycetes or Basidiomycetes (Edgington, et al., 1971) making it desirable from a mycorrhizal standpoint;

3. Continuous usage may lead to establishment of tolerant strains of pathogens (Bartels-Schooley and MacNeill, 1971; Magie and Wilfret, 1974);

4. Absorption rates to soil particles depend on soil composition (Fuchs, et al., 1970; Schreiber, et al., 1971);

5. Since absorption is passive (Edgington, et al., 1971) benomyl must be made available in the root zone either by physical incorporation or by moving the chemical in solution down to the root zone;

6. If applied early to 1-0 stock with small root systems perhaps infection can be prevented until a certain amount of natural tolerance or resistance can be developed by the seedlings (Bloomberg, 1973); and,

7. Curative benefits might be obtained under proper conditions (Delp and Klopping, 1968).

This study was not designed to provide positive proof that one or more of the fungi isolated was the primary causal agent and thus, no direct attempt has been made to positively identify the causal agent(s) through Koch's postulates. The disease pattern, the high correlation between dead seedlings and isolation frequency of *Fusarium* spp. from them (with identifications verified by Dr. Snyder's laboratory, University of California), and the vast amount of literature dealing with disease-causing *Fusarium* spp. strongly implicates *Fusarium* spp. as the pathogens involved. However, the etiological role of soil conditions antecedent to buildup of these organisms may be a much more important factor, as is widely recognized in field crop disease work.

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APPENDIX I

INDIVIDUAL MORTALITY PLOT DATA

Section 31

Treatment rates (lbs/acre)	Bed 5 Plot 1 No.	Bed 5 Plot 1 %	Bed 5 Plot 2 No.	Bed 5 Plot 2 %	Bed 6 Plot 1 No.	Bed 6 Plot 1 %	Bed 6 Plot 2 No.	Bed 6 Plot 2 %	Bed 7 Plot 1 No.	Bed 7 Plot 1 %	Bed 7 Plot 2 No.	Bed 7 Plot 2 %	Bed 8 Plot 1 No.	Bed 8 Plot 1 %	Bed 8 Plot 2 No.	Bed 8 Plot 2 %	Bed 9 Plot 1 No.	Bed 9 Plot 1 %	Bed 9 Plot 2 No.	Bed 9 Plot 2 %	Percent sampling error[1]
First Treatment Evaluation																					
14	2	7.4	1	5.6	2	9.1	2	5.1	1	2.7	7	13.5	0	0	4	7.6	5	11.4	2	4.4	29
7	1	2.4	3	7.1	3	7.3	4	8.0	1	1.7	4	13.8	3	5.6	4	8.2	3	5.1	0	0	29
Check	4	12.1	7	21.9	1	2.5	2	3.9	1	2.1	7	17.1	5	11.6	3	7.3	3	7.3	4	9.1	45
Total mortality 89/1268 = 6.9%																					
Second Treatment Evaluation																					
42	0	0	-	2/	-	--	1	4.2	0	0	0	0	2	4.0	-	--	0	0	1	2.0	26
28	1	2.2	-	--	-	--	1	2.3	0	0	1	2.6	1	2.1	-	--	3	7.5	0	0	33
14	1	4.0	0	3/	0	0	0	3/0	0	0	1	3/2.2	0	3/ 0	0	0	1	2.6	0	3/0	12
7	3	7.5	0	0	0	2	4.4	0	0	1	4	1.0	2	0	0	5.0	8.9	0	0	0	24
Check	0	0	0	0	0	0	0	0	0	0	2	5.9	1	2.6	0	0	1	2.6	1	2.5	14

Total mortality 31/1765 = 1.8%

Total mortality for plots evaluated through both treatments 109/1268 = 8.6%

1/ Percent sampling error is defined as $S\bar{x}/n \times t.05 \times 100$ where $S\bar{x}$ = standard error of the mean, n = number of sample units, and t.05 = 95% confidence interval.

2/ 42 pounds, 28 pounds not added to these plots because of space limitations.

3/ Plots not receiving benomyl during second treatment.

INDIVIDUAL MORTALITY PLOT DATA (Continued)

Section 36

Treatment rates (lbs/acre)	Bed 5				Bed 6				Bed 7				Bed 8				Bed 9				Percent sampling error
	Plot 1		Plot 2		Plot 1		Plot 2		Plot 1		Plot 2		Plot 1		Plot 2		Plot 1		Plot 2		
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	
First Treatment Evaluation																					
14	2	3.1	0	0	0	0	0	0	0	0	1	2.4	1	2.0	5	8.5	1	1.9	1	2.0	18
7	3	5.0	2	3.4	2	4.0	0	0	0	0	0	0	0	0	4	6.9	0	0	2	2.6	18
Check	2	2.6	0	0	3	6.3	1	1.7	0	0	2	3.0	1	2.6	0	0	0	0	2	3.5	15
Total mortality 35/1621 = 2.2%																					
Second Treatment Evaluation																					
42	-	--	4	8.0	0	0	0	0	0	0	-	--	-	--	0	0	1	2.6	0	0	40
28	-	--	1	2.6	0	0	0	0	0	0	-	--	-	--	0	0	1	1.9	0	0	15
14	1	1.6	0	0	0	0	1	2.0	1	1.4	1	2.5	0	0	2	3.9	1	2.0	1	2.0	9
7	5	8.8	1	1.8	0	0	3	6.4	2	4.0	0	0	0	0	0	0	0	0	0	0	24
Check	2	2.7	0	0	2	4.4	0	0	0	0	1	1.6	0	0	0	0	0	0	0	0	11

Total mortality 31/2202 = 1.4%

Total mortality for plots evaluated through both treatments 59/1621 = 3.6%

INDIVIDUAL MORTALITY PLOT DATA (Continued)

Section 37

Treatment rates (lbs/acre)	Bed 1 Plot 1 No.	Bed 1 Plot 1 %	Bed 1 Plot 2 No.	Bed 1 Plot 2 %	Bed 2 Plot 1 No.	Bed 2 Plot 1 %	Bed 2 Plot 2 No.	Bed 2 Plot 2 %	Bed 3 Plot 1 No.	Bed 3 Plot 1 %	Bed 3 Plot 2 No.	Bed 3 Plot 2 %	Percent sampling error
First Treatment Evaluation													
14	1	2.1	1	1.5	0	0	5	12.5	2	5.1	5	12.8	121
7	4	7.4	2	5.3	1	2.4	4	11.1	4	16.0	9	12.3	87
Check	1	2.3	0	0	1	3.3	3	7.3	5	12.8	1	1.8	82
Total mortality 49/779 = 6.3%													
Second Treatment Evaluation													
42	-	--	1	2.6	0	0	0	0	1	1.4	0	0	29
28	-	--	0	0	0	0	0	0	3	4.1	0	0	46
14	3	6.4	1	1.5	1	4.4	2	5.7	1	2.7	0	0	44
7	0	0	0	0	0	0	1	3.2	0	0	2	3.1	28
Check	0	0	0	0	0	0	0	0	1	2.9	1	1.8	22

Total mortality 18/1244 = 1.5%

Total mortality for plots evaluated through both treatments 62/779 = 8.0%

APPENDIX II

FREQUENCY OF ISOLATION OF PERTINENT FUNGI FROM SEEDLINGS

Date	Seedling condition	Treatment	Seedlings cultured	*Cylindrocarpon*	*F. oxysporum*	*F. roseum*	*Fusarium* + *Cylindrocarpon*	% w/*Fusarium* or *Cylindrocarpon*	Competitive saprophytes 1/
Prespray	Healthy	No	9	1	5	6	7	78	0
	Top Dead	No	9	1	4	7	8	89	1
	Dead	No	8	0	3	4	5	63	0
4/30	Healthy	14	6	0	0	0	0	0	0
		7	6	0	0	0	0	0	0
		Ck	6	0	0	0	0	0	0
	Top Dead	14	5	0	2	1	3	60	3
		7	6	0	1	3	4	67	0
		Ck	7	2	2	1	4	57	1
5/15	Healthy	14	7	1	0	3	3	43	1
		7	8	2	0	0	2	25	2
		Ck	6	0	1	2	3	50	1
	Top Dead	14	6	0	2	3	3	50	0
		7	6	1	2	5	5	83	0
		Ck	6	1	3	3	5	83	0
	Dead	14	2	0	1	2	2	100	1
		7	1	1	1	1	1	100	0
		Ck	3	1	2	2	3	100	0
6/6	Healthy	14	8	0	0	1	1	12.5	1
		7	8	0	0	1	1	12.5	5
		Ck	8	0	0	1	1	12.5	5
	Dead	14	18	1	3	9	12	67	1
		7	7	0	2	4	7	100	1
		Ck	15	0	6	9	11	73	0

1/ *Trichoderma* sp., *Penicillium* spp., *Alternaria* spp., *Cladosporium* spp.

FREQUENCY OF ISOLATION OF PERTINENT FUNGI FROM SEEDLINGS, con.

Date	Seedling condition	Treatment	Seedlings cultured	*Cylindrocarpon*	*F. oxysporum*	*F. roseum*	*Fusarium* + *Cylindrocarpon*	% w/*Fusarium* or *Cylindrocarpon*	Competitive saprophytes 1/
6/29	Healthy	14	10	0	1	0	1	10	0
		7	10	0	0	0	0	0	0
		Ck	9	1	1	4	6	67	0
	Dead	14	13	0	5	10	12	92	1
		7	8	0	2	7	8	100	1
		Ck	8	0	4	7	8	100	0
7/30	Dead	14	18	0	0	14	14	78	0
		7	28	0	5	23	27	96	0
		Ck	20	0	1	19	20	100	0
8/14	Dead	42	7	0	4	3	6	86	0
		28	7	0	2	4	5	71	0
		14	12	0	4	9	9	75	1
		7	8	0	3	8	8	100	0
		Ck	9	0	3	6	8	89	0
9/13	Dead	42	3	0	3	2	3	100	0
		28	2	0	1	1	2	100	0
		14	5	0	1	3	4	80	0
		7	17	0	4	15	15	88	0
		Ck	6	0	2	5	5	83	0

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